LANGERHANS CELLS AND THE MODIFIED TECHNIC OF GOLD IMPREGNATION BY FERREIRA-MARQUES*

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The nature of the Langerhans cells (1) has long been a subject of controversy. Recent English and American authors (2, 3, 4) have followed the lead of Masson (5) and believe that the Langerhans cells are "effete" melanocytes in the process of exfoliation. On the other hand, continental European investigators (6, 7, 8) are traditionally of the opinion that they are nervous receptor cells.

A new point of view was introduced into the nervous theory by Ferreira-Marques (9) based on a new modification of gold impregnation technic. This method demonstrates a more complicated configuration of the Langerhans cells than the formerly used Gairns (10) or Cohnheim (11) methods. With the observations based exclusively on this method, Ferreira-Marques considers the cells to be modified Schwann cells in the epidermis and to have a direct connection with the cutaneous innervation. Thus, he established the hypothesis of the "intraepidermal sensory system." Recently Richter (12) employing this method, corroborated Feyrter (13) who interpreted chromophil cells in the cutis and epidermis as "intercalated" cells of the autonomic nervous system. These form a synapse between the peripheral and central parts.

The detailed structure of the Langerhans cells is believed only demonstrable with gold technic, however, the result of gold impregnation is notoriously inconsistent and variable (14, 15). Therefore, the nature of the cells is wide open for a personal interpretation. In our continuous study on the nature of the Langerhans cells, the various technics of gold impregnation have been used and it was found that the Ferreira-Marques modification yields a relatively consistent and clear cut result in showing cell morphology; its specificity, however, is thought yet to be determined. This report describes some of the results through the use of the Ferreira-Marques modification with special reference to the specificity of the technic in revealing the structure of the cells.

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MATERIALS AND METHODS

1. Materials. The skin of various parts of man, cats and guinea pigs was used. White, brown and black skin was used from each of these sources. When a control was used, intercostal muscles were excised from the animals and treated in a similar manner.

2. Methods. The impregnation was done according to the original description of Ferreira-Marques. The muscle tissues were stained with both the Ferreira-Marques and Gairns methods. These were examined without a dehydrating process, being teased manually to demonstrate the nerves.

OBSERVATIONS

The gold-impregnated cells were found in all of the materials used, including the ears of albino animals and "recessive white" areas of trunk skin. The structural detail of the cells coincided with that described by Ferreira-Marques. They were observed to have a clear eccentric nucleus and a blackish stained fibrillar cytoplasm. They were dendritic and devoid of pigment granules.

1. Epidermis

In the epidermis, nearly all of the cells were found above the basal layer (Fig. 1 a, d, e); however, some were located in between basal cells (Fig. 1 c). This finding was rather peculiar, as they are generally believed to hold a superficial position in the epidermis. However, an occurrence of the cells in the basal layer was discussed and thought plausible by Masson in view of his belief that the Langerhans cells were melanocytes free of pigment and that half "effete" melanocytes could be shown in this position.

The cells were provided with various types of processes insinuating between prickle cells. Some had contact with neighboring dendritic cells by extending their processes and appeared at times to show anastomoses. This was especially evident

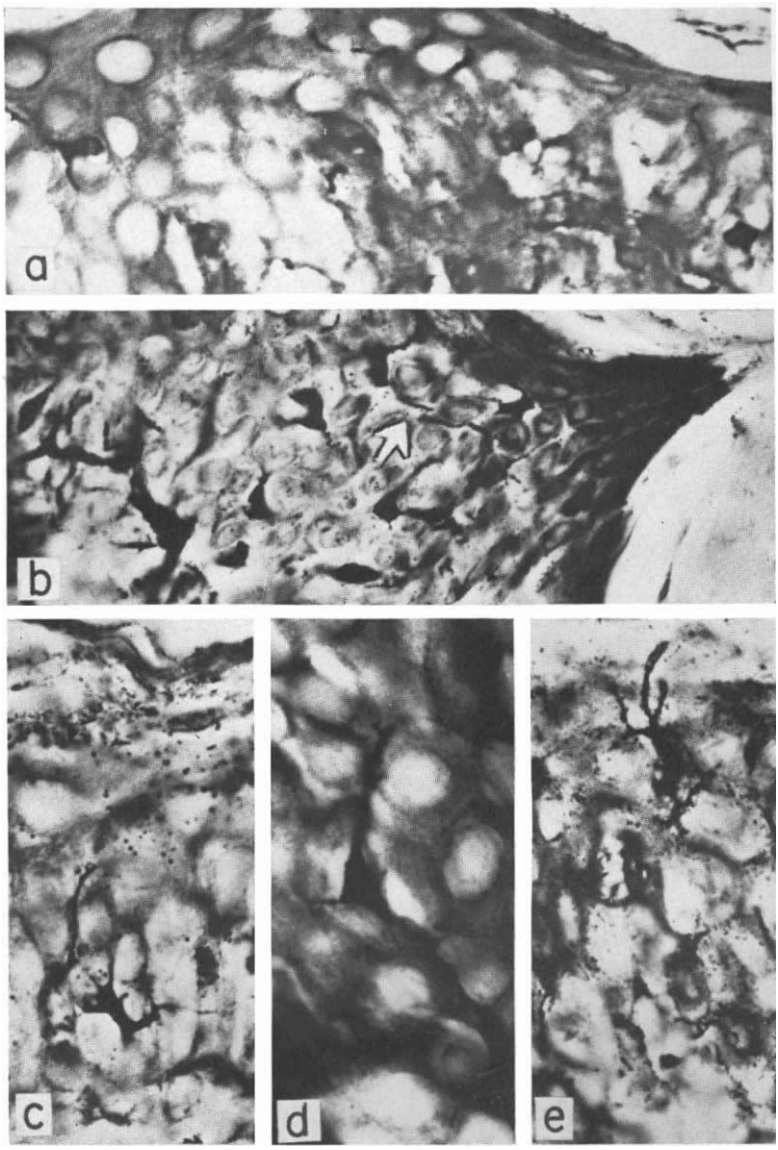

FIG. 1. a) Vertical section of Negro trunk skin, showing the superficial position of the Langerhans cells in the epidermis. 800 ×. b) Parallel cut section of cat sole showing the anastomosis of the cells (arrow). 800 ×. c–e) Vertical section of Negro trunk skin, showing the various position of the cells in the epidermis: c) in the basal layer, d) in the middle part of the Malpighian layer, e) in the more superficial layer, the processes reaching the horny layer. 1100 ×.

in the sections cut parallel to the surface (Fig. 1 b). The anastomosis of processes within the epidermis was described previously by Billingham *et al.* and Becker *et al.* In our preparations anastomoses were seen not only horizontally in the epidermis but also vertically between cells in the epidermis and dermis, as indicated in Figure 2. This feature was considered by Ferreira-Marques to be particularly significant. In the section illustrated in Figure 2 an anastomosis was found between the cells in the epidermis and a nerve "plexus" in the corium.

Melanocytes were impregnated (Fig. 3 a, b) occasionally. When impregnated, they seemed to be stained selectively appearing exactly in the same shape as when treated with the dopa reac-

tion (Fig. 3 b, d). In sections in which both Langerhans cells and melanocytes were demonstrated (Fig. 3 b, c) the cells in the superficial layer were strongly dendritic, whereas the melanocytes showed rudimentary processes.

The variable affinity of melanocytes toward gold was of interest. There has been some difference of opinion concerning this feature in the past (9). Miescher *et al* concluded that there was an essential distinction in the attitude toward metallic impregnation between the Langerhans cells and melanocytes and that the former alone were impregnable with gold in acid medium. Masson stated in support of their view that gold stained cytoplasm and processes of the cells while silver stained melanin after reduction; therefore, gold demonstrated only the Langerhans cells. On the other hand, Becker *et al* wrote that melanocytes could be shown differentially by gold after the sixth month of fetal life, confirming their view that Langerhans cells and melanocytes are essentially similar. In this regard, the present study suggests that gold impregnation of melanocytes is due to technical factors rather than to true affinity for gold. Melanocytes were shown only when either a strong reducing agent such as peroxide in the Ferreira-Marques method was used or the reduction was prolonged excessively for one reason or another.

2. Corium

There were numerous gold-impregnated cellular components in the corium (Fig. 4 a). These were located mostly in the papillary layer, particularly around the capillaries. As to the nature of these cells, Ferreira-Marques did not doubt that they were Schwann cells. We found their identification as a particular type of cell difficult, if not impossible, as they apparently included fibro-

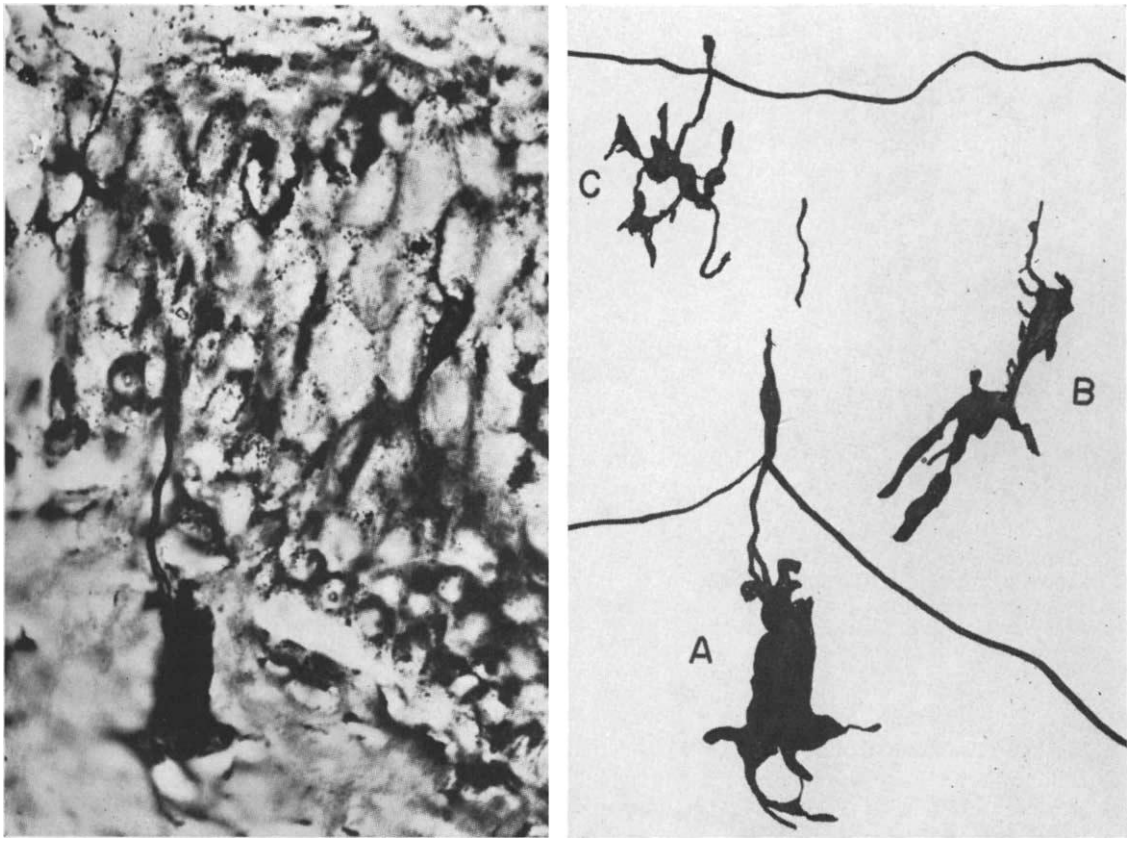

FIG. 2. Left, vertical section of Negro trunk skin; right, its schematic drawing to show the cell arrangement through the epidermis and dermis. 1100 ×. A) A fibrillar mass resembling a nerve plexus in the corium. B) and C) Langerhans cells in the epidermis. Note their fibrillar structure.

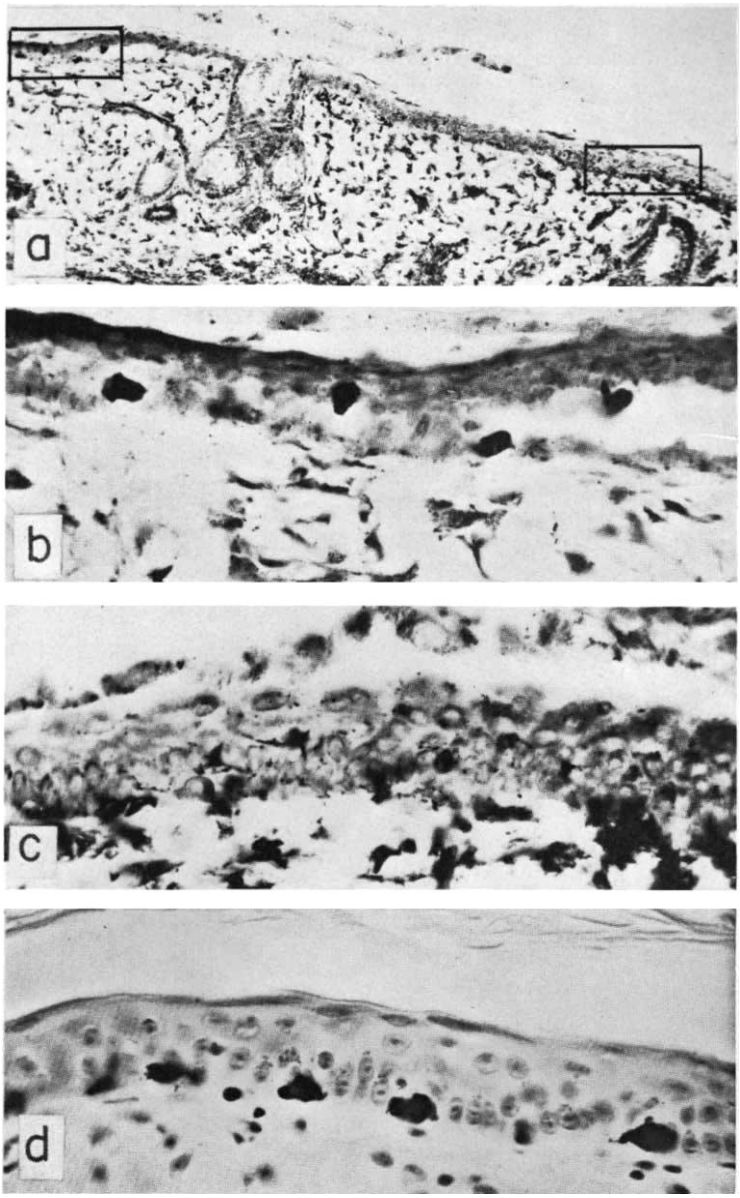

FIG. 3. Vertical sections of "recessive white" area of trunk skin of cat. a) Shows Langerhans cells and impregnated melanocytes in different areas. 90 ×. b) Views under higher magnification of the left square of the section a) in which melanocytes were impregnated. 400 ×. c) Shows the right square of the section in which the Langerhans cells were present, whereas melanocytes remained unstained. 400 ×. d) Shows the view of melanocytes in the neighboring section treated with the dopa reaction. 400 × Note the similarity of the shape to melanocytes stained by gold.

blasts, histiocytes and mast cells all of which have similar shape. Only when the section was cut parallel to the surface, some selectively impregnated cells were found to lie adjacent to the dermal-epidermal junction (Fig. 4 b). However, in nearly all such cases closer examination showed that these cells were Langerhans cells in the epidermis and were not situated on the dermal side of the junction. No really impregnated cells were even seen in the corium.

The nerve endings in the upper portion of the corium were frequently demonstrated (Fig. 4 c)

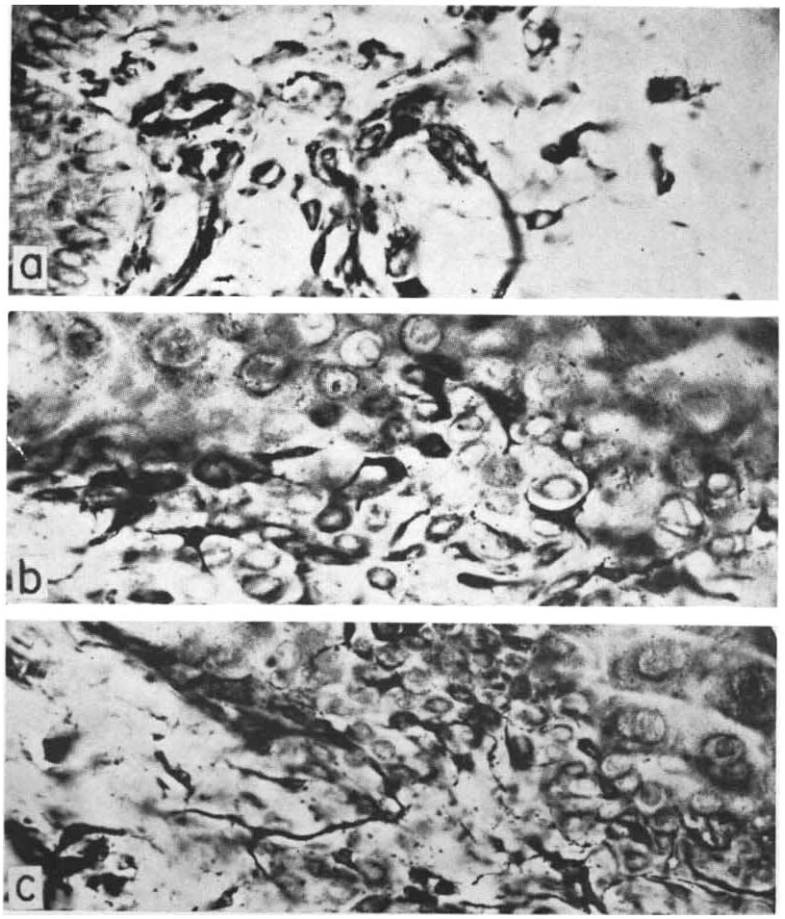

FIG. 4. Parallel cut sections of cat sole. a) Shows the various cellular components in the papilla impregnated in similar shape as the Langerhans cells. 400 ×. b) The Langerhans cells in the epidermis observed to lie immediately adjacent to the basal layer, due to the way in which the section was cut. 400 ×. c) The peripheral nerves in the papillary layer run over the epidermal cells. 400 ×.

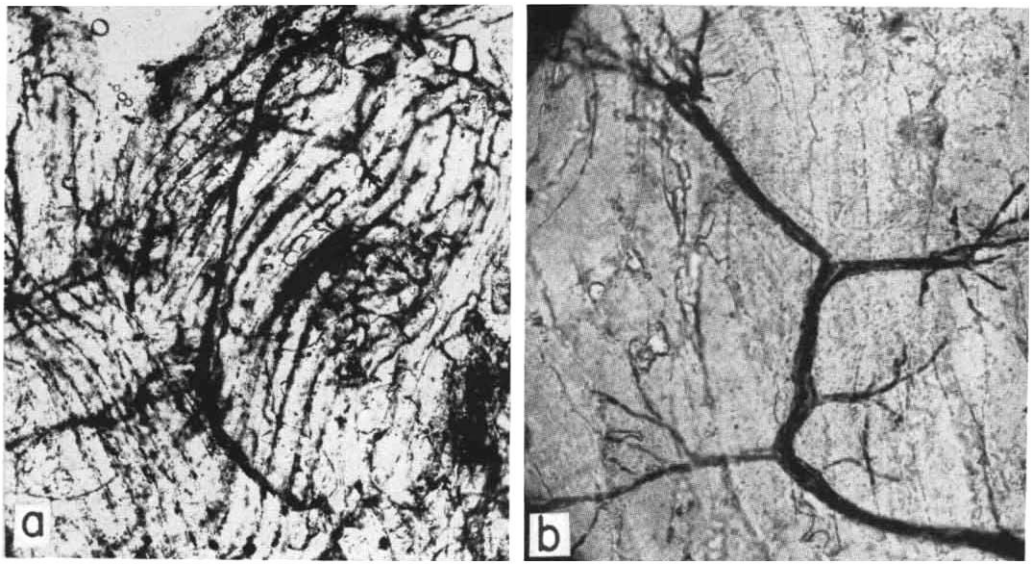

FIG. 5. The nerve endings in striated muscle of guinea pigs. a) Shows a nerve ending markedly obscured by nonspecific precipitate of metallic gold. 90 ×. b) Shows the structure of a nerve ending by the Gairns method. 90×.

and the observation confirmed the failure of finding them in the epidermis reported by Bazett *et al* (16), Woodlard (17) and Hagen *et al* (18). When they appeared to be in the epidermis, it was always possible to show their dermal position by examination of serial sections.

3. Muscles

The process of impregnation and reduction was best observed with the nerve endings in striated muscles. When a moderately weak reducing material, such as acetic acid or formic acid, was used, the nerve endings became visible slowly in from 6 hours to 18 hours (Fig. 5 b). With the Ferreira-Marques method in which a strong agent is used, the nerve endings were blackened almost instantly and an excessive precipitate in the muscular fibrillar spaces was so great that there was no differentiation of the nervous tissue (Fig. 5 a).

DISCUSSION AND CONCLUSION

The gold impregnation technic modified by Ferreira-Marques demonstrates some unique features of the Langerhans cells. Namely 1) a fine fibrillar structure of the cytoplasm, 2) complex branches, and 3) an occurrence of similar cells in the corium. Ferreira-Marques was further able to observe two anatomically distinctive components in the processes, continuity of a fibrillar cytoplasm of the cell, receptory process (Organe captativia), and receptory organ (Extensiones captationis) which had contact with the Schwann cells or nervous plexus in the corium. These peculiar features were accepted by him as a morphological reality and led him to interpret the Langerhans cells as an intraepidermal sensory unit responsible for reception of pain.

Since his observations and theory were based entirely on his own technic the accuracy and specificity of the technic must be discussed.

The principle of gold impregnation is based on the introduction of gold into a tissue followed by the reduction of it. This reaction gives rise to a peculiar color tint in a tissue that has an affinity for gold. Although the exact reaction is not known, the resultant product is presumed to be either sulfate, proteinate or metallic gold (Gomori (16)). According to Gray (15) the specific precipitate probably is not metallic gold. It seems then that gold impregnation demonstrates these tissues that form an unreactive compound with gold. However, this specific reaction is often obscured by a precipitate of metallic gold, which is a by-product of the reaction. Due to precipitation of metallic gold, there is often no differentiation of the Langerhans cells from artifacts. Nor is it possible to distinguish the true structure of the cells from artificial exaggeration. Miescher *et al* pointed out variability of the cell structure with reduction, stating that with a moderate reduction the Langerhans cells resembled melanocytes and with an excessive one a nervous element. Examination of striated muscle in the present study left little doubt that some of the characteristics of the Langerhans cells demonstrated by the Ferreira-Marques technic are the result of nonspecific precipitation of gold.

From this point of view, the Ferreira-Marques modification is not considered specific enough to reveal the true morphology of the Langerhans cells. Therefore, his nervous theory as to the nature of the Langerhans cells is not supported.

SUMMARY

1. The Ferreira-Marques modification of gold impregnation was used to demonstrate the Langerhans cells in the skin of man and animals.

2. This modification demonstrated the cells clearly. Nonspecific precipitate of metallic gold, however, was found to be considerable and obscured the true morphology of the cells.

3. Therefore, the hypothesis that these cells are the "intraepidermal sensory system" which was based exclusively on this impregnation technic is thought to require further confirmation.

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